Considered 11/21/02
MEC 8D15


```
=> FIL HCAPLUS
FILE 'HCAPLUS' ENTERED AT 17:52:19 ON 20 NOV 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)
```

Point of Contact:
Thomas G. Larson, Ph.D.
703-308-7309
CM1, Rm. 6 B 01


```
FILE COVERS 1907 - 20 Nov 2002  VOL 137 ISS 21
FILE LAST UPDATED: 19 Nov 2002  (20021119/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

CAS roles have been modified effective December 16, 2001. Please
check your SDI profiles to see if they need to be revised. For
information on CAS roles, enter HELP ROLES at an arrow prompt or use
the CAS Roles thesaurus (/RL field) in this file.

=> d que l33
L1         63443 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHORYLATION, BIOLOGICAL+NT
                 ,PFT/CT
L2          4358 SEA FILE=HCAPLUS ABB=ON  PLU=ON  DEPHOSPHORYLATION, BIOLOGICAL+
                 NT,PFT/CT
L3        101199 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT
L4          1333 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPEPTIDES+NT,PFT/CT
L5        102105 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 OR L4
L20        65285 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR L2
L22         3841 SEA FILE=HCAPLUS ABB=ON  PLU=ON  MICHAEL REACTION+PFT/CT
L25         1865 SEA FILE=HCAPLUS ABB=ON  PLU=ON  1,2-ETHANEDITHIOL/CT
L26           99 SEA FILE=HCAPLUS ABB=ON  PLU=ON  "THIOLS (ORGANIC) (L)
                 DITHIOLS"/CT
L29        13854 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L5 AND L20
L30       331072 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ISOTOPES+NT,PFT/CT
L31           12 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L29 AND L30
L32         5765 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L22 OR L25 OR L26
L33            1 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L31 AND L32


=> d que l34
L1         63443 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHORYLATION, BIOLOGICAL+NT
                 ,PFT/CT
L2          4358 SEA FILE=HCAPLUS ABB=ON  PLU=ON  DEPHOSPHORYLATION, BIOLOGICAL+
                 NT,PFT/CT
L3        101199 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT
L4          1333 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPEPTIDES+NT,PFT/CT
L5        102105 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 OR L4
L6         19810 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L-THREONINE/CT
L7         25642 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L-SERINE/CT
L8         31691 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L-TYROSINE/CT
L9         53676 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L6 OR L7 OR L8
```


Point of Contact:
Thomas G. Larson, Ph.D.
703-308-7309
CM1, Rm. 6 B 01

```
L10           1 SEA FILE=REGISTRY ABB=ON  PLU=ON  PHOSPHO-L-THREONINE/CN
L11         415 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L10
L12        1420 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHO-L-SERINE/CT
L13        1016 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHO-L-TYROSINE/CT
L14        2297 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L11 OR L12 OR L13
L15         640 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L14 AND L9
L16          88 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L15 AND L5
L17          78 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L16 AND L3
L20       65285 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR L2
L21          54 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L17 AND L20
L22        3841 SEA FILE=HCAPLUS ABB=ON  PLU=ON  MICHAEL REACTION+PFT/CT
L25        1865 SEA FILE=HCAPLUS ABB=ON  PLU=ON  1,2-ETHANEDITHIOL/CT
L26          99 SEA FILE=HCAPLUS ABB=ON  PLU=ON  "THIOLS (ORGANIC) (L)
                DITHIOLS"/CT
L32        5765 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L22 OR L25 OR L26
L34           1 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L21 AND L32


=> d que l36
L1         63443 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHORYLATION, BIOLOGICAL+NT
                ,PFT/CT
L2          4358 SEA FILE=HCAPLUS ABB=ON  PLU=ON  DEPHOSPHORYLATION, BIOLOGICAL+
                NT,PFT/CT
L3        101199 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT
L4          1333 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPEPTIDES+NT,PFT/CT
L5        102105 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 OR L4
L6         19810 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L-THREONINE/CT
L7         25642 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L-SERINE/CT
L8         31691 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L-TYROSINE/CT
L9         53676 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L6 OR L7 OR L8
L10           1 SEA FILE=REGISTRY ABB=ON  PLU=ON  PHOSPHO-L-THREONINE/CN
L11         415 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L10
L12        1420 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHO-L-SERINE/CT
L13        1016 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHO-L-TYROSINE/CT
L14        2297 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L11 OR L12 OR L13
L15         640 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L14 AND L9
L16          88 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L15 AND L5
L17          78 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L16 AND L3
L20       65285 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR L2
L21          54 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L17 AND L20
L35       56124 SEA FILE=HCAPLUS ABB=ON  PLU=ON  MASS SPECTROMETRY+NT,PFT/CT
L36           2 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L21 AND L35


=> d que l39
L1         63443 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHORYLATION, BIOLOGICAL+NT
                ,PFT/CT
L2          4358 SEA FILE=HCAPLUS ABB=ON  PLU=ON  DEPHOSPHORYLATION, BIOLOGICAL+
                NT,PFT/CT
L3        101199 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT
L4          1333 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPEPTIDES+NT,PFT/CT
L5        102105 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 OR L4
L20        65285 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR L2
L30       331072 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ISOTOPES+NT,PFT/CT
L35        56124 SEA FILE=HCAPLUS ABB=ON  PLU=ON  MASS SPECTROMETRY+NT,PFT/CT
L37        13854 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L5 AND L20
L38           12 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L37 AND L30
L39            1 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L38 AND L35
```

```
=> d que l41
L1        63443 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHORYLATION, BIOLOGICAL+NT
                ,PFT/CT
L2         4358 SEA FILE=HCAPLUS ABB=ON  PLU=ON  DEPHOSPHORYLATION, BIOLOGICAL+
                NT,PFT/CT
L3       101199 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT
L4         1333 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPEPTIDES+NT,PFT/CT
L5       102105 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 OR L4
L20       65285 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR L2
L22        3841 SEA FILE=HCAPLUS ABB=ON  PLU=ON  MICHAEL REACTION+PFT/CT
L25        1865 SEA FILE=HCAPLUS ABB=ON  PLU=ON  1,2-ETHANEDITHIOL/CT
L26          99 SEA FILE=HCAPLUS ABB=ON  PLU=ON  "THIOLS (ORGANIC) (L)
                DITHIOLS"/CT
L30      331072 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ISOTOPES+NT,PFT/CT
L32        5765 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L22 OR L25 OR L26
L37       13854 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L5 AND L20
L38          12 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L37 AND L30
L41           1 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L38 AND L32


=> d que l44
L1        63443 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHORYLATION, BIOLOGICAL+NT
                ,PFT/CT
L2         4358 SEA FILE=HCAPLUS ABB=ON  PLU=ON  DEPHOSPHORYLATION, BIOLOGICAL+
                NT,PFT/CT
L3       101199 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT
L4         1333 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PHOSPHOPEPTIDES+NT,PFT/CT
L5       102105 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 OR L4
L20       65285 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR L2
L37       13854 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L5 AND L20
L42         905 SEA FILE=HCAPLUS ABB=ON  PLU=ON  AFFINITY LABELING+NT,PFT/CT
L44           3 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L37 AND L42


=> s l33 or l34 or l36 or l39 or l41 or l44
L45           5 L33 OR L34 OR L36 OR L39 OR L41 OR L44

=> FIL MEDLINE
FILE 'MEDLINE' ENTERED AT 13:02:38 ON 21 NOV 2002

 FILE LAST UPDATED: 20 NOV 2002 (20021120/UP). FILE COVERS 1958 TO DATE.

 On June 9, 2002, MEDLINE was reloaded. See HELP RLOAD for details.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2002 vocabulary. Enter HELP THESAURUS for details.

 If you received SDI results from MEDLINE on October 8, 2002, these may
 have included old POPLINE data and in some cases duplicate abstracts.
 For further information on this situation, please visit NLM at:
 http://www.nlm.nih.gov/pubs/techbull/so02/so02_popline.html

 To correct this problem, CAS will remove the POPLINE records from the
 MEDLINE file and process the SDI run dated October 8, 2002 again.

 Customers who received SDI results via email or hard copy prints on
 October 8, 2002 will not be charged for this SDI run. If you received
 your update online and displayed answers, you may request a credit by
 contacting the CAS Help Desk at 1-800-848-6533 in North America or
 614-447-3698 worldwide, or via email to help@cas.org
```

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> d que l52
L46        64404 SEA FILE=MEDLINE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L47        52645 SEA FILE=MEDLINE ABB=ON  PLU=ON  "SPECTRUM ANALYSIS, MASS"+NT,P
                 FT/CT
L48         7420 SEA FILE=MEDLINE ABB=ON  PLU=ON  AFFINITY LABELS+NT,PFT/CT
L51          616 SEA FILE=MEDLINE ABB=ON  PLU=ON  L46 AND L47
L52            2 SEA FILE=MEDLINE ABB=ON  PLU=ON  L48 AND L51


=> d que l53
L46        64404 SEA FILE=MEDLINE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L47        52645 SEA FILE=MEDLINE ABB=ON  PLU=ON  "SPECTRUM ANALYSIS, MASS"+NT,P
                 FT/CT
L49         9901 SEA FILE=MEDLINE ABB=ON  PLU=ON  ISOTOPE LABELING+NT,PFT/CT
L51          616 SEA FILE=MEDLINE ABB=ON  PLU=ON  L46 AND L47
L53            3 SEA FILE=MEDLINE ABB=ON  PLU=ON  L51 AND L49


=> d que l57
L46        64404 SEA FILE=MEDLINE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L47        52645 SEA FILE=MEDLINE ABB=ON  PLU=ON  "SPECTRUM ANALYSIS, MASS"+NT,P
                 FT/CT
L51          616 SEA FILE=MEDLINE ABB=ON  PLU=ON  L46 AND L47
L54        59500 SEA FILE=MEDLINE ABB=ON  PLU=ON  ETHYLENE GLYCOL/CT OR
                 GLYCOLS+NT/CT
L55            6 SEA FILE=MEDLINE ABB=ON  PLU=ON  L51 AND L54
L56        47963 SEA FILE=MEDLINE ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT OR
                 PHOSPHOPEPTIDES+NT,PFT/CT
L57            2 SEA FILE=MEDLINE ABB=ON  PLU=ON  L56 AND L55


=> d que l59
L46        64404 SEA FILE=MEDLINE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L47        52645 SEA FILE=MEDLINE ABB=ON  PLU=ON  "SPECTRUM ANALYSIS, MASS"+NT,P
                 FT/CT
L51          616 SEA FILE=MEDLINE ABB=ON  PLU=ON  L46 AND L47
L54        59500 SEA FILE=MEDLINE ABB=ON  PLU=ON  ETHYLENE GLYCOL/CT OR
                 GLYCOLS+NT/CT
L55            6 SEA FILE=MEDLINE ABB=ON  PLU=ON  L51 AND L54
L58        37364 SEA FILE=MEDLINE ABB=ON  PLU=ON  THREONINE/CT OR SERINE/CT OR
                 TYROSINE/CT
L59            2 SEA FILE=MEDLINE ABB=ON  PLU=ON  L55 AND L58


=> d que l61
L48         7420 SEA FILE=MEDLINE ABB=ON  PLU=ON  AFFINITY LABELS+NT,PFT/CT
L49         9901 SEA FILE=MEDLINE ABB=ON  PLU=ON  ISOTOPE LABELING+NT,PFT/CT
L56        47963 SEA FILE=MEDLINE ABB=ON  PLU=ON  PHOSPHOPROTEINS+NT,PFT/CT OR
                 PHOSPHOPEPTIDES+NT,PFT/CT
L60           50 SEA FILE=MEDLINE ABB=ON  PLU=ON  L56 AND L49
L61            0 SEA FILE=MEDLINE ABB=ON  PLU=ON  L60 AND L48


=> s l52 or l53 or l57 or l59 or l61
L62            9 L52 OR L53 OR L57 OR L59 OR L61
```

— should be diethyl

— should be diethyl

Searched by Thom Larson, STIC, 308-7309

```
=> FIL EMBASE
FILE 'EMBASE' ENTERED AT 13:40:45 ON 21 NOV 2002
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 FILE COVERS 1974 TO 14 Nov 2002 (20021114/ED)

 EMBASE has been reloaded. Enter HELP RLOAD for details.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> d que l69
L63         9930 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L64         4064 SEA FILE=EMBASE ABB=ON  PLU=ON  DEPHOSPHORYLATION+NT,PFT/CT
L65        13613 SEA FILE=EMBASE ABB=ON  PLU=ON  L63 OR L64
L66        61867 SEA FILE=EMBASE ABB=ON  PLU=ON  MASS SPECTROMETRY+NT,PFT/CT
L67          153 SEA FILE=EMBASE ABB=ON  PLU=ON  L65 AND L66
L68          458 SEA FILE=EMBASE ABB=ON  PLU=ON  EHTYLENE GLYCOL/CT OR GLYCOL+NT
                 ,PFT/CT OR DIOL/CT OR ALKANEDIOL+NT,PFT/CT
L69            0 SEA FILE=EMBASE ABB=ON  PLU=ON  L67 AND L68


=> d que l73
L63         9930 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L64         4064 SEA FILE=EMBASE ABB=ON  PLU=ON  DEPHOSPHORYLATION+NT,PFT/CT
L65        13613 SEA FILE=EMBASE ABB=ON  PLU=ON  L63 OR L64
L66        61867 SEA FILE=EMBASE ABB=ON  PLU=ON  MASS SPECTROMETRY+NT,PFT/CT
L67          153 SEA FILE=EMBASE ABB=ON  PLU=ON  L65 AND L66
L70       406525 SEA FILE=EMBASE ABB=ON  PLU=ON  ISOTOPE+NT,PFT/CT
L71            6 SEA FILE=EMBASE ABB=ON  PLU=ON  L67 AND L70
L72        68784 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHOPROTEIN+NT,PFT/CT OR
                 PROTEIN PHOSPHORYLATION+NT,PFT/CT
L73            2 SEA FILE=EMBASE ABB=ON  PLU=ON  L71 AND L72


=> d que l76
L63         9930 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L64         4064 SEA FILE=EMBASE ABB=ON  PLU=ON  DEPHOSPHORYLATION+NT,PFT/CT
L65        13613 SEA FILE=EMBASE ABB=ON  PLU=ON  L63 OR L64
L66        61867 SEA FILE=EMBASE ABB=ON  PLU=ON  MASS SPECTROMETRY+NT,PFT/CT
L67          153 SEA FILE=EMBASE ABB=ON  PLU=ON  L65 AND L66
L72        68784 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHOPROTEIN+NT,PFT/CT OR
                 PROTEIN PHOSPHORYLATION+NT,PFT/CT
L74        21966 SEA FILE=EMBASE ABB=ON  PLU=ON  ISOTOPE LABELING+NT,PFT/CT
L75            8 SEA FILE=EMBASE ABB=ON  PLU=ON  L67 AND L74
L76            4 SEA FILE=EMBASE ABB=ON  PLU=ON  L75 AND L72


=> d que l78
L63         9930 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
L64         4064 SEA FILE=EMBASE ABB=ON  PLU=ON  DEPHOSPHORYLATION+NT,PFT/CT
L65        13613 SEA FILE=EMBASE ABB=ON  PLU=ON  L63 OR L64
L66        61867 SEA FILE=EMBASE ABB=ON  PLU=ON  MASS SPECTROMETRY+NT,PFT/CT
L67          153 SEA FILE=EMBASE ABB=ON  PLU=ON  L65 AND L66
L77          347 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHO AMINO ACID+NT,PFT/CT
L78            3 SEA FILE=EMBASE ABB=ON  PLU=ON  L67 AND L77


=> d que l81
L63         9930 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHORYLATION+NT,PFT/CT
```

{should be ethiol instead of diol — I'll redo this part and bring it to you ASAP

```
L64         4064 SEA FILE=EMBASE ABB=ON  PLU=ON  DEPHOSPHORYLATION+NT,PFT/CT
L65        13613 SEA FILE=EMBASE ABB=ON  PLU=ON  L63 OR L64
L66        61867 SEA FILE=EMBASE ABB=ON  PLU=ON  MASS SPECTROMETRY+NT,PFT/CT
L67          153 SEA FILE=EMBASE ABB=ON  PLU=ON  L65 AND L66
L72        68784 SEA FILE=EMBASE ABB=ON  PLU=ON  PHOSPHOPROTEIN+NT,PFT/CT OR
                 PROTEIN PHOSPHORYLATION+NT,PFT/CT
L79         8923 SEA FILE=EMBASE ABB=ON  PLU=ON  AFFINITY LABELING+NT,PFT/CT OR
                 AFFINITY CHROMATOGRAPHY+NT,PFT/CT
L80            2 SEA FILE=EMBASE ABB=ON  PLU=ON  L67 AND L79
L81            1 SEA FILE=EMBASE ABB=ON  PLU=ON  L80 AND L72


=> s l69 or l73 or l76 or l78 or l81
L82            8 L69 OR L73 OR L76 OR L78 OR L81

=> FIL BIOSIS
FILE 'BIOSIS' ENTERED AT 14:02:18 ON 21 NOV 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 20 November 2002 (20021120/ED)


=> d que l89
L83       120540 SEA FILE=BIOSIS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT
                 ?
L84        74519 SEA FILE=BIOSIS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L85         5116 SEA FILE=BIOSIS ABB=ON  PLU=ON  ISOTOP? (5A) (LABEL? OR TAG?)
L86         1102 SEA FILE=BIOSIS ABB=ON  PLU=ON  L83 AND L84
L87           10 SEA FILE=BIOSIS ABB=ON  PLU=ON  L86 AND L85
L88        12128 SEA FILE=BIOSIS ABB=ON  PLU=ON  PHOSPHOPROTEIN OR PHOSPHO
                 PROTEIN OR PHOSPHOPEPTIDE OR PHOSPHO PEPTIDE
L89            2 SEA FILE=BIOSIS ABB=ON  PLU=ON  L87 AND L88


=> d que l91
L83       120540 SEA FILE=BIOSIS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT
                 ?
L84        74519 SEA FILE=BIOSIS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L85         5116 SEA FILE=BIOSIS ABB=ON  PLU=ON  ISOTOP? (5A) (LABEL? OR TAG?)
L86         1102 SEA FILE=BIOSIS ABB=ON  PLU=ON  L83 AND L84
L87           10 SEA FILE=BIOSIS ABB=ON  PLU=ON  L86 AND L85
L90        44250 SEA FILE=BIOSIS ABB=ON  PLU=ON  PROTEIN (5A) PHOSPHOR?
L91            6 SEA FILE=BIOSIS ABB=ON  PLU=ON  L87 AND L90


=> d que l94
L83       120540 SEA FILE=BIOSIS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT
                 ?
L84        74519 SEA FILE=BIOSIS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L86         1102 SEA FILE=BIOSIS ABB=ON  PLU=ON  L83 AND L84
L93         1021 SEA FILE=BIOSIS ABB=ON  PLU=ON  ETHANE DITHIOL OR ETHANEDITHIOL
                  OR DITHIOL
L94            2 SEA FILE=BIOSIS ABB=ON  PLU=ON  L86 AND L93


=> d que l102
```

L ok

```
L83      120540 SEA FILE=BIOSIS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT
                ?
L84       74519 SEA FILE=BIOSIS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L86        1102 SEA FILE=BIOSIS ABB=ON  PLU=ON  L83 AND L84
L95        6751 SEA FILE=BIOSIS ABB=ON  PLU=ON  PHOSPHOSERINE OR PHOSPHOTYROSIN
                E OR PHOSPHOTHREONINE
L96         441 SEA FILE=BIOSIS ABB=ON  PLU=ON  PHOSPHO SERINE OR PHOSPHO
                TYROSINE OR PHOSPHO THREONINE
L97        6993 SEA FILE=BIOSIS ABB=ON  PLU=ON  L95 OR L96
L98          95 SEA FILE=BIOSIS ABB=ON  PLU=ON  L86 AND L97
L101      43923 SEA FILE=BIOSIS ABB=ON  PLU=ON  ISOTOP?
L102          1 SEA FILE=BIOSIS ABB=ON  PLU=ON  L98 AND L101


=> s l89 or l91 or l94 or l102
L103          9 L89 OR L91 OR L94 OR L102

=> FIL WPIDS
FILE 'WPIDS' ENTERED AT 14:16:13 ON 21 NOV 2002
COPYRIGHT (C) 2002 THOMSON DERWENT

FILE LAST UPDATED:          20 NOV 2002      <20021120/UP>
MOST RECENT DERWENT UPDATE:     200275        <200275/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> PATENT IMAGES AVAILABLE FOR PRINT AND DISPLAY >>>

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    SEE http://www.derwent.com/dwpi/updates/dwpicov/index.html <<<

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    PLEASE VISIT:
 http://www.stn-international.de/training_center/patents/stn_guide.pdf <<<

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    GUIDES, PLEASE VISIT:
    http://www.derwent.com/userguides/dwpi_guide.html <<<

=> d que l116
L104        3254 SEA FILE=WPIDS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT?

L105        6587 SEA FILE=WPIDS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L106          24 SEA FILE=WPIDS ABB=ON  PLU=ON  L104 AND L105
L109         213 SEA FILE=WPIDS ABB=ON  PLU=ON  PHOSPHOSERINE OR PHOSPHOTYROSINE
                  OR PHOSPHOTHREONINE
L110          45 SEA FILE=WPIDS ABB=ON  PLU=ON  PHOSPHO SERINE OR PHOSPHO
                TYROSINE OR PHOSPHO THREONINE
L111         234 SEA FILE=WPIDS ABB=ON  PLU=ON  L109 OR L110
L116           1 SEA FILE=WPIDS ABB=ON  PLU=ON  L106 AND L111


=> d que l118
L104        3254 SEA FILE=WPIDS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT?

L105        6587 SEA FILE=WPIDS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L106          24 SEA FILE=WPIDS ABB=ON  PLU=ON  L104 AND L105
L113        1223 SEA FILE=WPIDS ABB=ON  PLU=ON  ETHANEDITHIOL OR ALKYLDITHIOL
                OR DITHIOL
L118           0 SEA FILE=WPIDS ABB=ON  PLU=ON  L106 AND L113
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=> d que l122
L104       3254 SEA FILE=WPIDS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT?

L105       6587 SEA FILE=WPIDS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L106         24 SEA FILE=WPIDS ABB=ON  PLU=ON  L104 AND L105
L119      10214 SEA FILE=WPIDS ABB=ON  PLU=ON  THREONINE OR SERINE OR TYROSINE

L120          7 SEA FILE=WPIDS ABB=ON  PLU=ON  L106 AND L119
L121      23225 SEA FILE=WPIDS ABB=ON  PLU=ON  AFFIN?
L122          2 SEA FILE=WPIDS ABB=ON  PLU=ON  L120 AND L121


=> d que l123
L104       3254 SEA FILE=WPIDS ABB=ON  PLU=ON  PHOSPHORYLAT? OR DEPHOSPHORYLAT?

L105       6587 SEA FILE=WPIDS ABB=ON  PLU=ON  MASS (2A) SPECTRO?
L106         24 SEA FILE=WPIDS ABB=ON  PLU=ON  L104 AND L105
L107        763 SEA FILE=WPIDS ABB=ON  PLU=ON  ISOTOP? (5A) (LABEL? OR TAG?)
L108          5 SEA FILE=WPIDS ABB=ON  PLU=ON  L106 AND L107
L121      23225 SEA FILE=WPIDS ABB=ON  PLU=ON  AFFIN?
L123          3 SEA FILE=WPIDS ABB=ON  PLU=ON  L108 AND L121


=> s l116 or l118 or l122 or l123
L125          5 L116 OR L118 OR L122 OR L123



=> dup rem l62 l37 l103 l82 l125
FILE 'MEDLINE' ENTERED AT 14:31:34 ON 21 NOV 2002

FILE 'HCAPLUS' ENTERED AT 14:31:34 ON 21 NOV 2002
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FILE 'WPIDS' ENTERED AT 14:31:34 ON 21 NOV 2002
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PROCESSING COMPLETED FOR L62
PROCESSING COMPLETED FOR L37
PROCESSING COMPLETED FOR L103
PROCESSING COMPLETED FOR L82
PROCESSING COMPLETED FOR L125
L126         35 DUP REM L62 L37 L103 L82 L125 (1 DUPLICATE REMOVED)

=> D IBIB ABS 1-35

L126 ANSWER 1 OF 35  HCAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2002:142913  HCAPLUS
DOCUMENT NUMBER:          136:196180
TITLE:                    Detecting enzyme activity in an immunoassay using
                          protein or peptide substrate
```

INVENTOR(S): Kraemer, Joachim; Mander, Thomas; Peiker, Christine; Henco, Karsten
PATENT ASSIGNEE(S): Evotec Biosystems A.-G., Germany
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002014543	A2	20020221	WO 2001-EP9354	20010813
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1182263	A1	20020227	EP 2000-117457	20000811
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
EP 1199370	A1	20020424	EP 2000-122707	20001018
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
EP 1217078	A1	20020626	EP 2000-128176	20001221
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
AU 2001093768	A5	20020225	AU 2001-93768	20010813

PRIORITY APPLN. INFO.:
EP 2000-117457 A 20000811
EP 2000-118431 A 20000824
EP 2000-122707 A 20001018
EP 2000-128176 A 20001221
WO 2001-EP9354 W 20010813

AB The invention relates to a process for detecting enzyme activity in an immunoassay, in particular to a process for detecting dephosphorylation of phosphoserine or phosphothreonine by the activity of a protein phosphatase as well as to a process for detecting acetyltransferase or deacetylase activity in an immunoassay. The immunoassay comprises the following steps: (a) providing a protein, a peptide, or a deriv. thereof comprising the sequence motif -Z-X-Y- or -Y-X-Z- wherein Z = an amino acid to be modified by the enzyme, X = a sequence of amino acids, preferably between 0 and 1000 amino acids which may be the same or different, Y = a discrimination enhancer for the binding to on antibody, as a substrate for the enzyme; (b) incubating the protein, peptide, or deriv. thereof with the enzyme to form a modified protein, peptide, or deriv. thereof; (c) adding an antibody discriminating the modified Z from the unmodified Z position of said protein, peptide, or deriv. thereof, said discrimination being mediated by the presence of the affinity enhancer; and (d) detecting the enzyme activity. The invention relates further to a kit for carrying out the assay and to a luminescently labeled ligand. The assay could be used for screening modulators for enzyme activity.

L126 ANSWER 2 OF 35 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:142912 HCAPLUS
DOCUMENT NUMBER: 136:196179
TITLE: Detection of serine/threonine kinase activity in an

immunoassay using a pre-phosphorylated substrate and application to drug screening
INVENTOR(S): Kraemer, Joachim; Mander, Thomas; Bethell, Richard; Benson, Neil; Boyd, Helen; Greengrass, Pam; Kinloch, Ross
PATENT ASSIGNEE(S): Evotec Biosystems A.-G., Germany; Pfizer Limited
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002014542	A2	20020221	WO 2001-EP9186	20010808
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1182263	A1	20020227	EP 2000-117457	20000811
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 2002012133	A5	20020225	AU 2002-12133	20010808

PRIORITY APPLN. INFO.: EP 2000-117457 A 20000811
WO 2001-EP9186 W 20010808

AB The present invention describes a process for detecting threonine or serine kinase activity in an immunoassay using a pre-phosphorylated substrate. The invention further relates to a kit for carrying out the assay and to a luminescently labeled ligand. The process of the present invention as well as the kit and the labeled ligand may be used for screening for specific modulators of serine or threonine kinase activity.

L126 ANSWER 3 OF 35 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:658659 HCAPLUS
DOCUMENT NUMBER: 137:197868
TITLE: Phosphoprotein binding agents and methods of their use
INVENTOR(S): Goshe, Michael B.; Conrads, Thomas P.; Veenstra, Timothy D.; Panisko, Ellen A.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002119505	A1	20020829	US 2001-788286	20010216
WO 2002066988	A2	20020829	WO 2002-US4564	20020215
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				

PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: US 2001-788286 A 20010216

AB The invention provides reagents and methods for characterizing (i.e., identification and/or quantitation) the phosphorylation states of proteins. Proteins may be post-transcriptionally modified such that they contain phosphate groups at either some or all of their serine, threonine, tyrosine, histidine, and/or lysine amino acid residues. In many cases the extent to which a protein is phosphorylated dets. its bioactivity, i.e., its ability to effect cell functions such as differentiation, division, and metab. Hence, a powerful tool for diagnosing various diseases and for furthering the understanding of protein-protein interactions is provided. Two equal .beta.-casein samples were labeled with ethanedithiol (EDT) or EDT-2H4, resp., under .beta.-elimination conditions with NaOH. The labeled samples were quenched, desalted, denatured, reduced, biotinylated with iodoacetyl-PEO-biotin, and digested with trypsin. The labeled peptides were purified by affinity chromatog. using immobilized avidin and analyzed capillary reversed-phase liq. chromatog.-mass spectrometry.

L126 ANSWER 4 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:348154 BIOSIS
DOCUMENT NUMBER: PREV200200348154
TITLE: Method for the comparative quantitative analysis of proteins and other biological material by **isotopic labeling** and **mass spectroscopy**.
AUTHOR(S): Chait, Brian T. (1); Cowburn, David; Oda, Yoshi
CORPORATE SOURCE: (1) N.Y., NY USA
ASSIGNEE: The Rockefeller University
PATENT INFORMATION: US 6391649 May 21, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (May 21, 2002) Vol. 1258, No. 3, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

AB The present invention is a method for accurately comparing the levels of cellular components, such as proteins, present in samples which differ in some respect from each other using **mass spectroscopy** and **isotopic labeling.** A first sample of biological matter, such as cells, is cultured in a first medium and a second sample of the same biological matter is cultured in a second medium, wherein at least one isotope in the second medium has a different abundance than the abundance of the same isotope in the first medium. One of the samples is modulated, such as by treatment with a bacteria, a virus, a drug, hormone, a chemical or an environmental stimulus. The samples are combined and at least one protein is removed. The removed protein is subjected to **mass spectroscopy** to develop a mass spectrum. A ratio is computed between the peak intensities of at least one closely spaced pair of peaks to determine the relative abundance of the protein in each sample. The protein is identified by the mass spectrum or through other techniques known in the art. Modifications to the **proteins**, such as the **phosphorylation** of the **protein**, and the site of the modification may also be determined through the process of the present invention. The method is applicable to the components of any type of biological matter which are ionizable and may therefore be analyzed by **mass spectroscopy**.

L126 ANSWER 5 OF 35 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-682774 [73] WPIDS
DOC. NO. NON-CPI: N2002-539050
DOC. NO. CPI: C2002-192635
TITLE: Reagent for **mass spectrometric** analysis of proteins for determining **phosphorylation** state of proteins, for screening therapeutics that alter **phosphorylation** state of protein and as diagnostic for detecting diseases.
DERWENT CLASS: B04 D16 S03 V05
INVENTOR(S): CONRADS, T P; GOSHE, M B; PANISKO, E A; VEENSTRA, T D
PATENT ASSIGNEE(S): (CONR-I) CONRADS T P; (GOSH-I) GOSHE M B; (PANI-I) PANISKO E A; (VEEN-I) VEENSTRA T D; (BATT) BATTELLE MEMORIAL INST
COUNTRY COUNT: 100
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002066988	A2	20020829	(200273)*	EN	46

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW

US 2002119505 A1 20020829 (200273)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002066988	A2	WO 2002-US4564	20020215
US 2002119505	A1	US 2001-788286	20010216

this applicn.

PRIORITY APPLN. INFO: US 2001-788286 20010216
AN 2002-682774 [73] WPIDS
AB WO 200266988 A UPAB: 20021113
NOVELTY - A reagent (I) for **mass spectrometric** analysis of proteins that satisfies the general formula (F1) or (F2).
DETAILED DESCRIPTION - A reagent (I) for **mass spectrometric** analysis of proteins that satisfies the general formula (F1) or (F2).
(I) satisfies the general formula B-L-PhRG (F1), where B is a binding agent that selectively binds to a capture reagent (CR), L is a linker group that comprises at least one isotopically heavy atom and a **phosphorylation** reactive group (PhRG) that selectively labels proteins at one or more residues that were formerly occupied by phosphate group, or satisfies the general formula B-B1-X1-(CH2)n-(X2-(CH2)m)x-X3-(CH2)p-X4-B2-PhRG (F2), where B is a binding agent, PhRG is a phosphate reactive group, B1-X1-(CH2)n-(X2-(CH2)m)x-X3-(CH2)p-X4-B2 is a linker group, where X1, X2, X3 and X4, are independently chosen from O, S, NH, NR, NRR1+, CO, COO, COS, S-S, SO, SO2, CO-NR, CS-NR1, Si-O, aryl, or diaryl, where at least one of the X1, X2, X3 and X4 groups comprises an isotopically heavy atom.
USE - (I) is useful for comparing the **phosphorylation** states of one or more proteins in two or more samples, involves providing a substantially chemically identical and differentially

isotopically labeled protein reactive reagent (I) for each sample, reacting each sample with (I) to provide protein bound to (I), where such bound proteins are differentially **labeled** with stable **isotopes**, capturing bound proteins of the samples using the capture reagent that selectively binds the binding agent, releasing captured bound proteins from the capture reagent by disrupting the interaction between the binding agent and the capture reagent, and detecting the released bound proteins.The bound proteins in the samples are enzymatically or chemically processed to convert them into bound peptides. The protein portion of one or more of the bound proteins are sequenced by tandem **mass spectrometry** to identify the bound protein.

The amount of one or more **phosphorylated** proteins in the sample is determined by **mass spectrometry** and further involves introducing into a sample a known amount of one or more internal standards for each protein to be quantified. The **phosphorylated** amino acid residues are **threonine, serine** and **tyrosine**. The released bound proteins are separated by chromatography prior to detecting the bound proteins by **mass spectrometry**. Number of proteins in a single sample are detected and identified or all of the proteins in a sample are identified. The relative amounts of one or more proteins in two or more samples are determined and further involves combining differentially labeled samples, capturing bound proteins from the combined samples and measuring relative abundances of the bound proteins differentially labeled proteins. The proteins quantified are membrane proteins. The different samples contain proteins originating from different organelles or different subcellular fractions or represents proteins expressed in response to different environmental or nutritional conditions, different chemical or physical stimuli or at different times, or proteins expressed in different disease states. (I) is useful for screening a therapeutic that alters a **phosphorylation** state of a protein, involves contacting at least one test sample containing the protein with the therapeutic, providing at least one control sample containing the protein, removing one or more phosphate groups from one or more amino acid residues of the protein in the test sample and control sample, tagging the test sample and the control sample with (I), and detecting the level of **phosphorylation** of tagged proteins in the test sample and the control sample, and determining whether the therapeutic altered the level of **phosphorylation** of the tagged proteins in the test sample.

(I) is useful for detecting more than one type of **phosphorylated** amino acid residue in a protein, involves removing the phosphate group from at least one **serine** residue or at least one **threonine** residue, removing the phosphate group from at least one **tyrosine** residue, tagging the **serine** residue or **tyrosine** residue with (I), tagging the **tyrosine** residue with (I) and detecting the tagged protein. Removing the phosphate group from **serine** residue or **threonine** residue is after the removal of phosphate group from **tyrosine**. Tagging **serine** residue or **threonine** residue is done after tagging the **tyrosine** residue (all claimed). (I) is useful for characterization of **phosphorylation** state of multiple proteins i.e., useful to profile the **phosphorylation** state of multiple proteins from tissue samples such as tumor samples, body fluids such as urine, saliva or blood, or cell cultures, as diagnostic for the detection of diseases associated with hyper- or hypo-**phosphorylation** of protein, for screening to identify compounds that affect the **phosphorylation** state of protein i.e., to identify potential therapeutic agent to alter the **phosphorylation** state of proteins suspected of contributing to disease, and for measuring absolute

quantitative amount of proteins in sample. (I) is useful for diagnosing various diseases and for understanding protein-protein interaction and for identifying and/or detecting number of proteins in a single sample. (I) is useful as a diagnostic tool to identify subjects suffering from diseases caused by protein **phosphorylation** abnormalities.

ADVANTAGE - (I) is applied to peptides that are generated via enzymatic or chemical processing or is applied to proteins followed by protein sequencing. By using (I), the **phosphorylation** state of a specific protein is compared with a control sample without the need for protein sequencing, quantification or the use of antibodies selective for the **phosphorylated** protein itself.

Dwg.0/6

L126 ANSWER 6 OF 35 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-304072 [34] WPIDS
CROSS REFERENCE: 2002-089807 [12]
DOC. NO. NON-CPI: N2002-237932
DOC. NO. CPI: C2002-088421
TITLE: Detecting multiple analytes by separating a set of reporter signals having common property from molecules lacking common property, altering signal, detecting and distinguishing altered forms of signal from each other.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): CHAIT, B T; KERSHNAR, E R; LATIMER, D R; LIZARDI, P M; MATTESSICH, M J; MCCONNEL, K J; MORROW, J S; ROTH, M E
PATENT ASSIGNEE(S): (AGIL-N) AGILIX CORP
COUNTRY COUNT: 95
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002014867	A2	20020221	(200234)*	EN	341

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2001083562 A 20020225 (200245)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002014867	A2	WO 2001-US41709	20010813
AU 2001083562	A	AU 2001-83562	20010813

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001083562	A Based on	WO 200214867

PRIORITY APPLN. INFO: US 2001-283498P 20010412; US 2000-224939P 20000811
AN 2002-304072 [34] WPIDS
CR 2002-089807 [12]
AB WO 200214867 A UPAB: 20020717
NOVELTY - Detecting (M1) multiple analytes involves separating a set of reporter signals (RS), where each RS has a common property, from molecules

lacking the common property, altering the RS, and detecting and distinguishing the altered forms of the RS from each other.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a set of RS (I) comprising a number of RS, where the RS have a common property, allowing the RS to be distinguished or separated from molecules lacking this property, the RS can be altered, and be distinguished from every other altered form of RS;

(2) kits comprising a set of reporter molecules (RM), where

(a) each RM comprises an RS and a decoding tag, where each RM comprises a different decoding tag and RS, and a set of coding molecules comprising a specific binding molecule interacting with a different analyte, and coding tag interacting with a different decoding tag; or

(b) or an RS and a coupling tag, where the RM comprises a different coupling tag and different RS;

(3) a set of labeled proteins (LPs) (II) where each LP comprises a protein or peptide and RS attached to the protein or peptide, where alteration of the RS alters the LPs, and altered forms of each LP can be distinguished from every other altered form of LP;

(4) a labeled protein (III) where the LP comprises a protein or peptide and a RS attached to the protein or peptide, where the LP has a common property, and the common property allows the LP to be distinguished or separated from molecules lacking the common property, the RS can be altered, and alteration of the RS alters the LP, where altered form of the LP can be distinguished from the unaltered form of LP;

(5) detecting (M2) a protein, by detecting a LP, where the LP comprises a protein or peptide and a RS attached to the protein or peptide, where the LP is altered by altering the RS, detecting an altered form of the LP, where the LP is altered by altering the RS, and identifying the protein based on the characteristics of the LP and altered form of the LP;

(6) a catalog (IV) of proteins and peptides comprises proteins and peptides in a sample detected by M1;

(7) producing (M3) a protein signature;

(8) analyzing (M3) a protein sample, by mixing a protein sample with a predetermined amount of RS calibrator, where the protein sample has a known amount of protein and comprises a target protein fragment, the target protein fragment and the RS calibrator can be altered, where the altered form of the RS calibrator can be distinguished from the unaltered form of the target protein fragment, altering the target protein fragment and RS calibrator, and detecting the altered forms of the target protein fragment and RS calibrator;

(9) a set of RS calibrators (V);

(10) a kit for producing a protein signature comprising (V) and one or more reagents for treating a protein sample to produce protein fragments;

(11) a mixture (VI) comprising (V) and a set of target protein fragments;

(12) a set of target protein fragments (VII);

(13) a set of nucleic acid molecules (VIII) where each nucleic acid molecule comprises a nucleotide segment encoding an amino acid segment comprising a RS peptide and a protein or peptide of interest;

(14) a set of amino acid segments (IX) where each amino acid segment comprises a RS peptide and a protein or peptide of interest;

(15) a cell (X) comprising (VIII);

(16) a set of cells (XI) comprising a nucleic acid molecule where each nucleic acid molecule comprises a nucleotide segment encoding an amino acid segment comprising a RS peptide and a protein or peptide of interest;

(17) an organism (XII) comprising (VIII);

(18) a set of organisms (XIII) each organism comprising a nucleic acid molecule where each nucleic acid molecule comprises a nucleotide segment encoding an amino acid segment comprising a RS peptide and a protein or peptide of interest;

(19) detecting (M4) expression involves detecting a target altered RS peptide derived from one or more expression samples, where the expression samples collectively comprise (VIII);

(20) detecting (M5) cells involves detecting a target altered RS peptide derived from one or more (X);

(21) detecting (M6) cell samples by detecting a target altered RS peptide derived from one or more cell samples; and

(22) detecting (M7) organisms by detecting a target altered RS peptide derived from one or more (XII).

USE - The method (M1) is useful for detecting multiple analytes (claimed). M1 is useful for detection of analytes and biomolecules, (such as proteins, peptides and protein fragments), preferably for multiplex detection and analysis of analytes and biomolecules. M1 is useful to detect a specific analyte (in a specific sample or in multiple samples) or multiple analytes (in a single sample or multiple samples), and to gather and catalog information about unknown analytes. M1 is useful as a detection system in a number of fields, including antibody or protein microarrays, DNA microarrays, expression profiling, comparative genomics, immunology, diagnostic assay and quality control. M1 is useful as a detection and analysis system for protein analysis, proteome analysis, proteomic, protein expression profiling, de novo protein discovery, functional genomics and protein detection.

ADVANTAGE - M1 increases the sensitivity and accuracy of detection of analytes of interest, and allows a complex sample of analytes to be quickly and easily cataloged in a reproducible manner. M1 is compatible with techniques involving cleavage, treatment or fragmentation of a bulk sample in order to simplify the sample prior to introduction into the first stage of a multistage detection system. M1 is also compatible with any desired sample, including raw extracts and fractionated samples.

Dwg.0/10

L126 ANSWER 7 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2002357917 EMBASE
TITLE: Mapping of phosphorylated proteins on two-dimensional polyacrylamide gels using protein phosphatase.
AUTHOR: Yamagata A.; Kristensen D.B.; Takeda Y.; Miyamoto Y.; Okada K.; Inamatsu M.; Yoshizato K.
CORPORATE SOURCE: Dr. K. Yoshizato, Developmental Biology Laboratory, Graduate School of Science, Hiroshima University, 1-3-1, Kagamiyama, Higashihiroshima, Hiroshima 739-8526, Japan. kyoshiz@hiroshima-u.ac.jp
SOURCE: Proteomics, (1 Sep 2002) 2/9 (1267-1276).
Refs: 34
ISSN: 1615-9853 CODEN: PROTC7
COUNTRY: Germany
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB This study developed an enzymatic method for high-throughput mapping of phosphoproteins on two-dimensional (2-D) polyacrylamide gels. Proteins of cultured rat skin fibroblasts were divided into two aliquots, one of which was dephosphorylated using recombinant lambda protein phosphatase and the other was not treated with the enzyme. The two aliquots were then subjected to 2-D electrophoresis. Phosphoproteins could be mapped on the 2-D gel of the nontreated aliquot by comparing the gels of the two

aliquots, because the phosphoproteins in the treated aliquot shifted to more basic positions on the gel. This technique revealed that approximately 5% of the detectable proteins were phosphorylated. Fourteen phosphoproteins were identified by mass spectrometry, including proteasome component C8 and small glutaminerich tetratricopeptide repeat-containing protein. Furthermore, the extent of phosphorylation of two actin modulating proteins, destrin and cofilin, was found to be significantly reduced when the cells were chemically or enzymatically detached from the culture dishes. The method developed by this study can generally be applied to all biological materials and is useful for high-throughput mapping of phosphoproteins in proteome research.

L126 ANSWER 8 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 2002:328907 BIOSIS
DOCUMENT NUMBER: PREV200200328907
TITLE: Analysis of **protein phosphorylation** using **mass spectrometry**: Deciphering the phosphoproteome.
AUTHOR(S): Mann, Matthias (1); Ong, Shao-En (1); Gronborg, Mads (1); Steen, Hanno (1); Jensen, Ole N. (1); Pandey, Akhilesh (1)
CORPORATE SOURCE: (1) Center for Experimental Bioinformatics, University of Southern Denmark, Odense M, DK-5230: mann@bmb.sdu.dk, pandey@cebi.sdu.dk Denmark
SOURCE: Trends in Biotechnology, (June, 2002) Vol. 20, No. 6, pp. 261-268. http://journals.bmn.com/journals/list/latest?jcode=tibtech. print.
ISSN: 0167-7799.
DOCUMENT TYPE: Article
LANGUAGE: English
AB In signal transduction in eukaryotes, **protein phosphorylation** is a key event. To understand signaling processes, we must first acquire an inventory of **phosphoproteins** and their **phosphorylation** sites under different conditions. Because **phosphorylation** is a dynamic process, elucidation of signaling networks also requires quantitation of these **phosphorylation** events. In this article, we outline several methods for enrichment of **phosphorylated proteins** and peptides and discuss various options for their identification and quantitation with special emphasis on **mass spectrometry**-based techniques.

L126 ANSWER 9 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:322616 BIOSIS
DOCUMENT NUMBER: PREV200200322616
TITLE: Quantitative proteome analysis: New technology and applications.
AUTHOR(S): Aebersold, R. (1); Lee, Hookeun (1); Han, David; Wright, Michael (1); Zhou, Huilin (1); Griffin, Tim (1); Purvine, Sam (1); Goodlett, David (1)
CORPORATE SOURCE: (1) Institute for Systems Biology, Seattle, WA USA
SOURCE: FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A12-A13. http://www.fasebj.org/. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Proteomics is a preferred functional genomics technology because its focus is proteins: the most significant class of molecules affecting biological

structure, function, and control. In this presentation we will discuss a new approach to quantitative proteome analysis and show results from selected applications of the technology to microbial and mammalian cell systems. The technology is based on a new class of chemical reagents termed **isotope** coded affinity **tags** (ICAT) (Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R, Nature Biotechnol 1999; 17:994-9). The reagents and the tandem **mass spectrometry** based analytical process allow the precise quantitation and identification of large numbers of proteins in complex mixtures rapidly and sensitively. The need to separate and analyze extremely complex peptide mixtures challenges the separation sciences. Optimized peptide separation protocols connected on-line with **mass spectrometers** will be discussed. The applications will document the performance of the method to examine changes in protein profile in yeast cells induced by metabolic shifts, to measure quantitative differences in the cell surface protein profile in mammalian cells, and to detect and quantify changes in **protein phosphorylation** profiles in cell lysates.

L126 ANSWER 10 OF 35 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-122223 [16] WPIDS
DOC. NO. NON-CPI: N2002-091676
DOC. NO. CPI: C2002-037465
TITLE: Selective labelling of phosphate groups in peptides and proteins for separation, isolation and detection of phosphoproteins and phosphopeptides, comprises the presence of carboxylic acids.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): AEBERSOLD, R; ZHOU, H
PATENT ASSIGNEE(S): (UNIW) UNIV WASHINGTON; (AEBE-I) AEBERSOLD R; (ZHOU-I) ZHOU H
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001096869	A1	20011220	(200216)*	EN	59

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2001066894 A 20011224 (200227)
US 2002049307 A1 20020425 (200233)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001096869	A1	WO 2001-US18988	20010612
AU 2001066894	A	AU 2001-66894	20010612
US 2002049307	A1 Provisional	US 2000-210972P	20000612
		US 2001-880713	20011018

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001066894	A Based on	WO 200196869

PRIORITY APPLN. INFO: US 2000-210972P 20000612; US 2001-880713 20011018
AN 2002-122223 [16] WPIDS
AB WO 200196869 A UPAB: 20020308
NOVELTY - Selective labelling phosphate groups in peptides or proteins in the presence of carboxylic acid groups, is new.
DETAILED DESCRIPTION - Selective labelling phosphate groups in peptides or proteins in the presence of carboxylic acid groups comprises:
(1) reacting the substrate to protect the phosphates as phosphoramides and the carboxylates as amides;
(2) selectively cleaving the phosphoramide bonds; and
(3) reacting the free phosphates with a label or tag.
INDEPENDENT CLAIMS are included for the following:
(1) detecting phosphopeptides in samples containing a mixture of peptides comprising:
(a) selective protection of carboxyl groups;
(b) selective labelling of phosphate groups; and
(c) detection of the labelled peptides;
(2) a kit for selectively labelling phosphopeptides in a mixture of peptides comprising:
(a) a protective group which reacts with a carboxylic acid or ester and a phosphate group; and
(b) a mild reagent for selectively regenerating any free phosphate groups in the peptide by reacting the protected peptides under mild acid conditions so that the phosphoramide bond is cleaved and the amide bonds is not cleaved.
USE - The new method is used for selectively labelling phosphate groups in peptides or proteins in the presence of carboxylic acid groups (claimed). It is useful in separation, isolation and detection of phosphoproteins and phosphopeptides.
Dwg.0/6

L126 ANSWER 11 OF 35 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-488919 [53] WPIDS
DOC. NO. NON-CPI: N2001-361715
DOC. NO. CPI: C2001-146870
TITLE: Assessing status of cellular pathway such as cell growth, cell death pathway, by applying cell lysate containing cellular pathway molecules to immobilized series of binding reagents which discriminate the molecules.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): DAY, A R; LIOTTA, L A; PAWLELETZ, K L; PETRICOIN, E F
PATENT ASSIGNEE(S): (IMMU-N) IMMUNOMATRIX INC
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001057530	A1	20010809	(200153)*	EN	56

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001033276 A 20010814 (200173)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001057530	A1	WO 2001-US3535	20010202
AU 2001033276	A	AU 2001-33276	20010202

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001033276	A Based on	WO 200157530

PRIORITY APPLN. INFO: US 2000-179997P 20000203
AN 2001-488919 [53] WPIDS
AB WO 200157530 A UPAB: 20010919
NOVELTY - Assessing (M1) the status of a selected signal transduction pathway (STP) in cells, comprises generating a cell lysate containing components of STP proteins, retaining STP molecules in states, applying lysate to an immobilized series of binding reagents (BR) which discriminate STP molecules, identifying binding events between STP molecules and BR and determining the state of the selected STP.

DETAILED DESCRIPTION - Assessing (M1) the status of a selected signal transduction pathway (STP) in cells, comprises generating a cell lysate containing components of STP proteins, retaining STP molecules in one or more states, applying lysate to an immobilized series of binding reagents (BR) which discriminate STP molecules, identifying binding events between STP molecules and BR and determining the state of the selected STP. The lysate contains components of STP proteins. The STP molecules are retained in one or more states such as inactive, activated, activity altered, **phosphorylated**, cleaved, modified and bound, which reflects signal transduction events taking place in the cells. The lysate is applied to immobilized BR and the state of STP is determined by identifying binding between the STP molecules and BR. INDEPENDENT CLAIMS are also included for the following:

(1) elucidating (M2) complex protein-protein interactions within defined protein pathways and protein networks in cells, by:

(a) generating a lysate of cells containing one or more protein components of the protein-protein interactions;

(b) applying the lysate to an immobilized series of BR which discriminate one or more protein components; and

(c) identifying binding events between the protein components and BR;

(2) a cellular STP profiling device (100) (D) for use in determining the presence of several analytes in a lysate of a sample of cells, comprising a porous support member (110) and many BR arranged and immobilized at several reaction sites on the support member, where the BR are selected and arranged to assess the status of a selected protein-protein interaction network when the lysate is applied on it;

(3) determining (M3) the state of a portion of a signal pathway or network in a cell, by:

(a) solubilizing the cellular contents;

(b) exposing the contents to a series of labeled BR to form a pattern of binding events; and

(c) analyzing the pattern of binding events;

(4) identifying (M4) proteins involved in cellular signaling or networks, by:

(a) exposing cells to one of a phosphatase inhibitor followed by a drug such that the **phosphorylation** state of one or more molecules is changed;

(b) analyzing patterns of groups of at least 2 **phosphorylated** molecules before and after exposure to the drug;

(c) exposing cells to a molecule which perturbs one or more pathways;

and

(d) comparing the patterns before and after exposure of the cells to the molecule; and

(5) identifying (M5) a repertoire of proteins that serve as acceptors for **phosphorylation**, by:

(a) treating a biological sample with one or more compounds that inhibit protein tyrosine and/or serine/threonine phosphatase activity;

(b) isolating and lyzing cells of interest;

(c) selecting and enriching for **phosphorylated** proteins using antibodies on an immobilized bait to produce an enriched fraction;

(d) separating the phosphoproteins in the enriched fraction;

(e) identifying a primary amino acid sequence of the separated proteins; and

(f) identifying binding molecules that specifically bind to the separated proteins.

USE - (M1) is useful for assessing the status of a signal transduction pathway such as cell growth, cell differentiation, cell death, cell movement, gene transcription regulation, hormonal autocrine or paracrine stimulation and cell adhesion pathways. Identifying (M4) proteins involved in cellular signaling or networks is useful for determining therapeutic efficacy and toxicity of a preselected molecule being evaluated for therapeutic potential. A cellular STP profiling device (D) is useful for determining the presence of several analytes in a cell lysate, to assess the status of a protein-protein interaction network i.e. STP relevant to a disease state, such as cancer, brain disease, cardiac disease or allergy. The immobilized BR quantitatively assesses the status of a series of STP proteins or other protein-protein interaction network, their **phosphorylated** or activated state and their binding partner, in a cell sample (claimed). Knowledge of the pathway status can be used by a diagnostician or clinician to determine the health of the sampled cells, drug or other treatment efficacy or toxicity, to identify candidates for selected therapies, to aid in therapy selection for a given subject and to determine drug toxicities.

DESCRIPTION OF DRAWING(S) - The figure shows the cellular signaling transduction pathway profiling device.

cellular signal transduction pathway profiling device 100

porous support member 110

Dwg.1/21

L126 ANSWER 12 OF 35 MEDLINE
ACCESSION NUMBER: 2001553854 MEDLINE
DOCUMENT NUMBER: 21486474 PubMed ID: 11502742
TITLE: Protein kinase C regulates the phosphorylation and cellular localization of occludin.
AUTHOR: Andreeva A Y; Krause E; Muller E C; Blasig I E; Utepbergenov D I
CORPORATE SOURCE: Forschungsinstitut fur Molekulare Pharmakologie, 13125 Berlin-Buch and Charite, Humboldt Universitat Berlin, 13092 Berlin, Germany.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Oct 19) 276 (42) 38480-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011016
Last Updated on STN: 20020122
Entered Medline: 20011204

AB Occludin is an integral membrane phosphoprotein specifically associated with tight junctions, contributing to the structure and function of this intercellular seal. Occludin function is thought to be regulated by phosphorylation, but no information is available on the molecular pathways involved. In the present study, the involvement of the protein kinase C pathway in the regulation of the phosphorylation and cellular distribution of occludin has been investigated. Phorbol 12-myristate 13-acetate and 1,2-dioctanoylglycerol induced the rapid phosphorylation of occludin in Madin-Darby canine kidney cells cultured in low extracellular calcium medium with a concomitant translocation of occludin to the regions of cell-cell contact. The extent of occludin phosphorylation as well as its incorporation into tight junctions induced by protein kinase C activators or calcium switch were markedly decreased by the protein kinase C inhibitor GF-109203X. In addition, in vitro experiments showed that the recombinant COOH-terminal domain of murine occludin could be phosphorylated by purified protein kinase C. Ser(338) of occludin was identified as an in vitro protein kinase C phosphorylation site using peptide mass fingerprint analysis and electrospray ionization tandem mass spectroscopy. These findings indicate that protein kinase C is involved in the regulation of occludin function at tight junctions.

L126 ANSWER 13 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:462073 BIOSIS
DOCUMENT NUMBER: PREV200100462073
TITLE: Selective analysis of phosphopeptides within a protein mixture by chemical modification, reversible biotinylation and **mass spectrometry**.
AUTHOR(S): Adamczyk, Maceij (1); Gebler, John C.; Wu, Jiang
CORPORATE SOURCE: (1) Department of Chemistry (9NM), Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL, 60064-6016: maciej.adamczyk@abbott.com USA
SOURCE: Rapid Communications in Mass Spectrometry, (2001) Vol. 15, No. 16, pp. 1481-1488. print.
ISSN: 0951-4198.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A new method combining chemical modification and affinity purification is described for the characterization of serine and threonine phosphopeptides in proteins. The method is based on the conversion of phosphoserine and phosphothreonine residues to S-(2-mercaptoethyl)cysteinyl or beta-methyl-S-(2-mercaptoethyl)cysteinyl residues by beta-elimination/1,2-**ethanedithiol** addition, followed by reversible biotinylation of the modified proteins. After trypsin digestion, the biotinylated peptides were affinity-isolated and enriched, and subsequently subjected to structural characterization by liquid chromatography/tandem **mass spectrometry** (LC/MS/MS). Database searching allowed for automated identification of modified residues that were originally **phosphorylated**. The applicability of the method is demonstrated by the identification of all known **phosphorylation** sites in a mixture of alpha-casein, beta-casein, and ovalbumin. The technique has potential for adaptations to proteome-wide analysis of protein **phosphorylation**.

ad 11/21/02

L126 ANSWER 14 OF 35 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:156255 HCAPLUS
DOCUMENT NUMBER: 134:322979
TITLE: Detection of tyrosine phosphorylated peptides by precursor ion scanning quadrupole TOF mass spectrometry in positive ion mode

Searched by Thom Larson, STIC, 308-7309

AUTHOR(S): Steen, Hanno; Kuester, Bernhard; Fernandez, Minerva; Pandey, Akhilesh; Mann, Matthias
CORPORATE SOURCE: Protein Interaction Laboratory at the Center for Experimental Bioinformatics Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, DK-5230, Den.
SOURCE: Analytical Chemistry (2001), 73(7), 1440-1448
CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Phosphorylation is a common form of protein modification. To understand its biol. role, the site of phosphorylation has to be detd. Generally, only limited amts. of phosphorylated proteins are present in a cell, thus demanding highly sensitive procedures for phosphorylation site detn. Here, a novel method is introduced which enables the localization of tyrosine phosphorylation in gel-sepd. proteins in the femtomol range. The method utilizes the immonium ion of phosphotyrosine at m/z 216.043 for pos. ion mode precursor ion scanning combined with the recently introduced Q2-pulsing function on quadrupole TOF mass spectrometers. The high resolving power of the quadrupole TOF instrument enables the selective detection of phosphotyrosine immonium ions without interference from other peptide fragments of the same nominal mass. Performing precursor ion scans in the pos. ion mode facilitates sequencing, because there is a no need for polarity switching or changing pH of the spraying solvent. Similar limits of detection were obtained in this approach when compared to triple-quadrupole mass spectrometers but with significantly better selectivity, owing to the high accuracy of the fragment ion selection. Synthetic phosphopeptides could be detected at 1 fmol/.mu.L, and 100 fmol of a tyrosine phosphorylated protein in gel was sufficient for the detection of the phosphorylated peptide in the unsepd. digestion mixt. and for unambiguous phosphorylation site detn. The new method can be applied to unknown protein samples, because the identification and localization of the modification is performed on the same sample.

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L126 ANSWER 15 OF 35 MEDLINE
ACCESSION NUMBER: 2001464890 MEDLINE
DOCUMENT NUMBER: 21401128 PubMed ID: 11509731
TITLE: Cell cycle regulation of myosin-V by calcium/calmodulin-dependent protein kinase II.
COMMENT: Comment in: Science. 2001 Aug 17;293(5533):1263-4
AUTHOR: Karcher R L; Roland J T; Zappacosta F; Huddleston M J; Annan R S; Carr S A; Gelfand V I
CORPORATE SOURCE: Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.
CONTRACT NUMBER: GM-52111 (NIGMS)
SOURCE: SCIENCE, (2001 Aug 17) 293 (5533) 1317-20.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20010820
Last Updated on STN: 20010910
Entered Medline: 20010906

AB Organelle transport by myosin-V is down-regulated during mitosis, presumably by myosin-V phosphorylation. We used mass spectrometry

phosphopeptide mapping to show that the tail of myosin-V was phosphorylated in mitotic Xenopus egg extract on a single serine residue localized in the carboxyl-terminal organelle-binding domain. Phosphorylation resulted in the release of the motor from the organelle. The phosphorylation site matched the consensus sequence of calcium/calmodulin-dependent protein kinase II (CaMKII), and inhibitors of CaMKII prevented myosin-V release. The modulation of cargo binding by phosphorylation is likely to represent a general mechanism regulating organelle transport by myosin-V.

L126 ANSWER 16 OF 35 MEDLINE
ACCESSION NUMBER: 2001690907 MEDLINE
DOCUMENT NUMBER: 21599443 PubMed ID: 11736647
TITLE: Ecto-protein kinase substrate p120 revealed as the cell-surface-expressed nucleolar phosphoprotein Nopp140: a candidate protein for extracellular Ca2+-sensing.
AUTHOR: Kubler D
CORPORATE SOURCE: German Cancer Research Center, Division of Pathochemistry B0100, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany.. d.kuebler@dkfz.de
SOURCE: BIOCHEMICAL JOURNAL, (2001 Dec 15) 360 (Pt 3) 579-87. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011213
Last Updated on STN: 20020206
Entered Medline: 20020205

AB A variety of cell membrane proteins become phosphorylated in their ecto-domains by cell-surface protein kinase (ecto-PK) activities, as detected in a broad spectrum of cell types. This study reports the isolation and identification of a frequent ecto-PK substrate, ecto-p120, using HeLa cells as a model. Data from MS and further biochemical and immunochemical means identified ecto-p120 as a cell-surface homologue of human nucleolar phosphoprotein p140 (hNopp140), which belongs to the family of argyrophilic (AgNOR-stainable) proteins. The superposition of (32)P-labelled ecto-nucleolar phosphoprotein p140 (ecto-Nopp140) with anti-Nopp140 immunostaining could be demonstrated in a wide range of cell lines without any exceptions, suggesting a nearly universal occurrence of cell-surface Nopp140. A previous, tentative association of ecto-p120 with the nucleoplasmic pre-mRNA-binding protein hnRNP U has thus been supplanted, since improved purification techniques have allowed unambiguous identification of this ecto-PK cell-surface substrate. Furthermore, we have shown that rapid suppression of ecto-hNopp140 phosphorylation resulted upon a rise in the free extracellular calcium, while lowering the calcium concentrations returned ecto-Nopp140 phosphorylation to the original level. It is important to note that these Ca(2+)-dependent effects on ecto-Nopp140 phosphorylation are not accompanied by alterations in the phosphorylation of other ecto-PK substrates. Our results indicate that, in addition to nucleolin, a further nucleolar protein, which was considered initially to be strictly intracellular, is identified as a cell-surface phosphoprotein.

L126 ANSWER 17 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:519503 BIOSIS
DOCUMENT NUMBER: PREV200100519503
TITLE: Analytical techniques from individual proteins to whole cells.

AUTHOR(S): Robinson, Carol V. (1); Cowburn, David
CORPORATE SOURCE: (1) Oxford Centre for Molecular Sciences, South Parks Road, Oxford, OX1 3QT: carolr@bioch.ox.ac.uk, david.cowburn@verizon.net UK
SOURCE: Current Opinion in Chemical Biology, (October, 2001) Vol. 5, No. 5, pp. 565-566. print.
ISSN: 1367-5931.
DOCUMENT TYPE: General Review
LANGUAGE: English
SUMMARY LANGUAGE: English

L126 ANSWER 18 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2001050365 EMBASE
TITLE: A multidimensional electrospray MS-based approach to phosphopeptide mapping.
AUTHOR: Annan R.S.; Huddleston M.J.; Verma R.; Deshaies R.J.; Carr S.A.
CORPORATE SOURCE: R.S. Annan, Dept. of Physical/Structural Chem., SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, United States. Roland_S_Annan@sbphrd.com
SOURCE: Analytical Chemistry, (1 Feb 2001) 73/3 (393-404).
Refs: 47
ISSN: 0003-2700 CODEN: ANCHAM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A new, multidimensional electrospray MS-based strategy for phosphopeptide mapping is described which eliminates the need to radiolabel protein with (32)P or (33)P. The approach utilizes two orthogonal MS scanning techniques, both of which are based on the production of phosphopeptide-specific marker ions at m/z 63 and/or 79 in the negative ion mode. These scan methods are combined with liquid chromatography - electrospray mass spectrometry and nanoelectrospray MS/MS to selectively detect and identify phosphopeptides in complex proteolytic digests. Low-abundance, low-stoichiometry phosphorylation sites can be selectively determined in the presence of an excess of nonphosphorylated peptides, even in cases where the signal from the phosphopeptide is indistinguishable from background in the conventional MS scan. The strategy, which has been developed and refined in our laboratory over the past few years, is particularly well suited to phosphoproteins that are phosphorylated to varying degrees of stoichiometry on multiple sites. Sensitivity and selectivity of the method are demonstrated here using model peptides and a commercially available phosphoprotein standard. In addition, the strategy is illustrated by the complete in vitro and in vivo phosphopeptide mapping of Sic1p, a regulator of the G1/S transition in budding yeast.

L126 ANSWER 19 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:146014 BIOSIS
DOCUMENT NUMBER: PREV200100146014
TITLE: On-membrane digestion of beta-casein for determination of **phosphorylation** sites by matrix-assisted laser desorption/ionization quadrupole/time-of-flight **mass spectrometry**.
AUTHOR(S): Lee, C. H.; McComb, M. E.; Bromirski, M.; Jilkine, A.; Ens, W.; Standing, K. G.; Perreault, H. (1)
CORPORATE SOURCE: (1) Department of Chemistry, University of Manitoba, Winnipeg, MB, R3T 2N2: perreau@cc.umanitoba.ca Canada

SOURCE: Rapid Communications in Mass Spectrometry, (2001) Vol. 15, No. 3, pp. 191-202. print.
ISSN: 0951-4198.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB This article discusses the features of a newly developed matrix-assisted laser desorption/ionization quadrupole/time-of-flight (MALDI-QqTOF) **mass spectrometer** that is useful in the analysis of **phosphorylated** peptides. Aliquots of beta-casein, a commonly used **phosphorylated** protein standard, were digested with trypsin directly on a non-porous polyurethane membrane used as sample support in MALDI-QqTOF **mass spectrometry** (MS) experiments. Although a complete peptide map was obtained, it was difficult to obtain sequence information for some of the tryptic fragments, in particular T1-2, which bears four phosphate groups and is thus difficult to ionize in positive mode. This article focuses on the sequencing of this particular fragment by comparing MS/MS spectra obtained using different precursor ions. These precursors associated with T1-2 were (M + H)+, (M + H)2+, and (M + H - nH3PO4)+ ions. Typically, **phosphorylated** ions showed facile unimolecular losses of phosphoric acid moieties, and produced limited backbone fragmentation. The abundance of (M + H)2+ ions of T1-2 in the full mass spectrum was low relative to that of (M + H)+. (M + H - 4H3PO4)+ ions as MS/MS precursors underwent backbone fragmentations, with **phosphoserine** residues transformed into dehydroalanines or serines. Unusual b + 18 u fragments were observed, although only for segments with previously **phosphorylated** serines. These partly interfered with c-ions, and were noticeable due to overlapping **isotopic** envelopes. It was possible to establish the sequence of **phosphorylated** tryptic fragment T1-2 and the location of phosphate groups using the mass of dehydroalanine residues (69 Da) and b + 18 u fragments as markers. All MS and MS/MS spectra obtained with fully **phosphorylated** beta-casein were compared with spectra acquired with **dephosphorylated** beta-casein obtained commercially. These comparisons helped assess the spectral differences caused by the presence of phosphate groups. Also, they highlighted the potential usefulness of conducting **dephosphorylation** directly on the probe prior to MALDI analysis in future studies.

L126 ANSWER 20 OF 35 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2001-007261 [01] WPIDS
DOC. NO. NON-CPI: N2001-005187
DOC. NO. CPI: C2001-001862
TITLE: Comparative quantitative analysis of post-translational changes in protein expression or any biological component which can be ionized by **isotopic labeling** and **mass spectroscopy**, useful for studying gene therapy effects.
DERWENT CLASS: B04 D16 J04 K08 S03
INVENTOR(S): CHAIT, B T; COWBURN, D; ODA, Y
PATENT ASSIGNEE(S): (UYRQ) UNIV ROCKEFELLER
COUNTRY COUNT: 21
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000067017	A1	20001109	(200101)*	EN	55
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: CA JP					
US 6391649	B1	20020521	(200239)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000067017	A1	WO 2000-US12026	20000503
US 6391649	B1	US 1999-304799	19990504

PRIORITY APPLN. INFO: US 1999-304799 19990504
AN 2001-007261 [01] WPIDS
AB WO 200067017 A UPAB: 20001230
NOVELTY - Comparing the relative abundance of, a protein (pro) in multiple samples (S) of biological matter (BM), a peptide (pep) derived from the same pro from multiple (S) of BM, an ionizable component of BM from multiple (S), a cellular component in multiple cell pools, BM from 2 animals or BM from 1 animal at different times by **mass spectroscopy** (MS) and isotropic labeling.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) comparing the relative abundance of a pro (M1) in multiple (S) of BM, comprises:

(a) culturing first and second (S) of BM in first and second mediums respectively (the first medium contains a natural abundance of isotopes (ISOs) and the second medium has the same ISOs as the first medium but in different abundances);

(b) modulating one of the (S);

(c) combining a portion of each of the (S);

(d) removing a pro from the combined (S);

(e) subjecting the removed pro to MS to develop a mass spectrum;

(f) computing a ratio between the peak intensities of a pair of closely spaced peaks; and

(g) determining the relative abundance of the pro in each (S) based on at least one computed ratio and then identifying the pro);

(2) comparing the relative abundance of a pep (M2) derived from the same pro from multiple (S) of BM, comprises:

(a) culturing a first cell pool in a first medium containing a natural abundance of ISOs;

(b) culturing a second cell pool in a second medium isotopically enriched in non-radioactive ISO;

(c) modulating one of the cell pools;

(d) combining a portion of each of the cell pools;

(e) extracting the pros from the combined cell pools;

(f) separating the extracted pros into several pros;

(g) digesting a pro of interest from the separated pros into several peps;

(h) subjecting the digested peps to MS to develop a mass spectrum; and

(i) determining the relative abundance of the pep from each (S) based on the mass spectrum;

(3) comparing relative abundance of an ionizable component of BM (M3) from multiple (S), comprises:

(a) culturing a first (S) of BM in first medium;

(b) culturing a second (S) of BM in a second medium in which the abundance of an ISO in the second medium is different to that in the first medium;

(c) modulating BM in one of the (S);

(d) combining a portion of each of the (S);

(e) subjecting a portion of the combined (S) to MS to develop a mass spectrum; and

(f) determining the relative abundance a component of interest in each (S) based on the mass spectrum;

(4) comparing the relative abundance of an ionizable cellular component (M4) in multiple cell pools, comprises:

(a) culturing a first cell pool in a first medium;

(b) culturing a second cell pool in a second medium which contains an ISO in a different abundance to that in the first medium;

(c) modulating one of the cell pools;

(d) combining a portion of each of the cell pools;

(e) removing a component of interest from the combined cell pools;

(f) subjecting the removed component of interest to MS to develop a mass spectrum;

(g) computing a ratio of the peak intensities of a pair of closely spaced peaks, corresponding to the component of interest of the mass spectrum; and

(h) identifying the component;

(5) comparing the relative abundance of BM from two animal subjects (M5) in which the BM can be analyzed by MS, comprises:

(a) feeding a first animal food;

(b) feeding a second animal food (including an ISO having a different abundance to that of the same ISO in the food fed to the second animal);

(c) withdrawing (S) of BM from first and second animals;

(d) combining a portion of the withdrawn BM from first and second animals;

(e) subjecting the combined BM to MS to develop a mass spectrum; and

(f) determining the relative abundance of a component of the combined BM based on the mass spectrum; and

(6) comparing the relative abundance of BM from a single animal (M6) at different time points, in which the BM can be analyzed by MS, comprises:

(a) withdrawing a first (S) of BM from the subject;

(b) feeding the subject food which includes an ISO having a different abundance to that of the same ISO in the food fed to the first animal, after withdrawing the first sample (sic);

(c) withdrawing the (S) BM from the subject after feeding;

(d) mixing portions of first and second (S);

(e) subjecting the combined (S) to MS to develop a mass spectrum; and

(f) determining the relative abundance of a component of the combined BM based on the mass spectrum.

USE - Comparing the relative abundance of, a pro in multiple (S) of BM, a pep derived from the same pro from multiple (S) of BM, an ionizable component of BM from multiple (S), a cellular component in multiple cell pools, BM from two animals or BM from a single animal at different times using MS and isotropic labeling (claimed).

The effective quantification of pro expression levels aids in understanding the interaction of gene expression with external factors in producing phenotypes and in understanding the molecular basis of physiological and pathological processes. Changes in the post-translational expression of pros with time may be studied by periodically withdrawing (S) from a control cell pool and a cell pool whose metabolism has been arrested, either one of which may be isotopically labeled. The effects of chemical compounds on the post-translational expression of pros in one cell pool may be compared with a cell pool which has not been exposed to the compound which is useful in screening drug candidates by giving an indication of side effects.

With a knowledge of the deleterious and positive changes in pro expression, the toxicology of pesticides, chemicals and environmental agents can also be examined in accordance with the method. In the field of agriculture, the effects of fertilizers, pesticides and pheromones on the

post-translational synthetic expression of pros can be studied. The effects of gene therapy can also be studied by the methods. The effects of treatments on the cell membranes themselves can also be studied in accordance with the method. The secreted by-products of the cell pools could also be compared by drawing (S) from the media of each pool, one of which is isotopically enriched, mixing the (S), removing the cells and any other unwanted components, and analyzing the remaining mixture of the (S) media by MS.

The post-translational effects of hormones, infectious agents such as viruses and bacteria, carcinogens, and trauma such as burns, can be similarly studied and quantified. Pain modulation can also be examined. The effects of cell differentiation in post-translational pro expression can also be studied by the methods.

DESCRIPTION OF DRAWING(S) - The figure shows a flow chart of the method for comparative quantitative analysis of post-translational changes in protein expression or any biological component which can be ionized by isotopic labeling and MS.

Dwg.3/13

L126 ANSWER 21 OF 35 MEDLINE
ACCESSION NUMBER: 2000309757 MEDLINE
DOCUMENT NUMBER: 20309757 PubMed ID: 10748151
TITLE: Sphingosine is a novel activator of 3-phosphoinositide-dependent kinase 1.
AUTHOR: King C C; Zenke F T; Dawson P E; Dutil E M; Newton A C; Hemmings B A; Bokoch G M
CORPORATE SOURCE: Departments of Immunology, Cell Biology, and Chemistry and the Skaggs Institute for Chemical Biology, Scripps Research Institute, La Jolla, California 92037, USA.
CONTRACT NUMBER: GM39434 (NIGMS)
GM43154 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jun 16) 275 (24) 18108-13.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20020420
Entered Medline: 20000720

AB 3-Phosphoinositide-dependent kinase 1 (PDK1) has previously been shown to phosphorylate the activation loop of several AGC kinase family members. In this study, we show that p21-activated kinase 1, the activity of which is regulated by the GTP-bound form of Cdc42 and Rac and by sphingosine, is phosphorylated by PDK1. Phosphorylation of p21-activated kinase 1 by PDK1 occurred only in the presence of sphingosine, which increased PDK1 autophosphorylation 25-fold. Sphingosine increased PDK1 autophosphorylation in a concentration-dependent manner and significantly increased phosphate incorporation into known PDK1 substrates. Studies on the lipid requirement for PDK1 activation found that both sphingosine isoforms and stearylamine also increased PDK1 autophosphorylation. However, C(10)-sphingosine, octylamine, and stearic acid were unable to increase PDK1 autophosphorylation, indicating that both a positive charge and a lipid tail containing at least a C(10)-carbon backbone were required for PDK1 activation. Three PDK1 autophosphorylation sites were identified after stimulation with sphingosine in a serine-rich region located between the kinase domain and the pleckstrin homology domain using two-dimensional phosphopeptide maps and matrix assisted laser desorption/ionization mass

spectroscopy. Increased phosphorylation of endogenous Akt at threonine 308 was observed in COS-7 cells expressing wild type PDK1, but not catalytically inactive PDK1, when cellular sphingosine levels were elevated by treatment with sphingomyelinase. Sphingosine thus appears to be a true PDK1 activator.

L126 ANSWER 22 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000106967 EMBASE
TITLE: Detailed analysis of the phosphorylation of the human La (SS-B) autoantigen. (De)phosphorylation does not affect its subcellular distribution.
AUTHOR: Broekhuis C.H.D.; Neubauer G.; Van der Heijden A.; Mann M.; Proud C.G.; Van Venrooij W.J.; Pruijn G.J.M.
CORPORATE SOURCE: G.J.M. Pruijn, Department of Biochemistry 161, University of Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, Netherlands. G.Pruijn@bioch.kun.nl
SOURCE: Biochemistry, (21 Mar 2000) 39/11 (3023-3033).
Refs: 73
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
031 Arthritis and Rheumatism
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The La (SS-B) autoantigen is an evolutionarily conserved phosphoprotein which plays an important role, most likely as an RNA chaperone, in various processes, such as the biosynthesis and maturation of RNA polymerase III transcripts in the cell nucleus and (internal) initiation of translation in the cytoplasm. In this study, the phosphorylation state of this protein from human HeLa and HEp-2 cells was characterized by high-resolution two-dimensional IEF/SDS-PAGE analysis, and phosphorylation sites were mapped by nanoelectrospray mass spectrometry. Furthermore, the effect of phosphorylation at the sites identified on the subcellular distribution of the protein was studied by site-directed mutagenesis. At least 14 isoelectric isoforms were discerned on 2-D gels with La protein from both types of cells. Metabolic labeling in combination with alkaline phosphatase treatment revealed that only a limited number of these isoforms could be attributed to phosphorylation. Four phosphorylation sites, Thr-302, Ser-325, Thr-362, and Ser-366, were mapped by mass spectrometric analysis of the isolated La protein from HeLa cells or the carboxy-terminal half of this protein. The analysis of mutants of La, in which the respective phosphorylated residues were replaced by either a neutral (alanine) or an acidic (aspartate) residue, by microinjection into Xenopus laevis oocytes on the one hand and transfection of HEp-2 cells on the other hand revealed that the subcellular distribution of this protein was not affected by these amino acid substitutions. These results strongly suggest that the signals that determine the subcellular distribution of this protein are not regulated by (de)phosphorylation of the target residues examined.

L126 ANSWER 23 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:465632 BIOSIS
DOCUMENT NUMBER: PREV200000465632
TITLE: Comparative quantification and identification of **phosphoproteins** using stable ~~isotope~~ **labeling** and liquid chromatography/**mass** ~~spectrometry~~.
AUTHOR(S): Weckwerth, Wolfram (1); Willmitzer, Lothar; Fiehn, Oliver

CORPORATE SOURCE: (1) Max-Planck-Institute of Molecular Plant Physiology, 14424, Potsdam Germany
SOURCE: Rapid Communications in Mass Spectrometry, (2000) Vol. 14, No. 18, pp. 1677-1681. print.
ISSN: 0951-4198.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A new liquid chromatography/**mass spectrometry** (LC/MS) method is described for relative quantification of **phosphoproteins** to simultaneously compare the **phosphorylation** status of **proteins** under two different conditions. Quantification was achieved by beta-elimination of phosphate from phospho-Ser/Thr followed by Micheal addition of ethanethiol and/or ethane-d5-thiol selectively at the vinyl moiety of dehydroalanine and dehydroamino-2-butyric acid. The method was evaluated using the model **phosphoprotein** alphaS1-casein, for which three **phosphopeptides** were found after tryptic digestion. Reproducibility of the relative quantification of seven independent replicates was found to be 11% SD. The dynamic range covered two orders of magnitude, and quantification was linear for mixtures of 0 to 100% alphaS1-casein and dephospho-alphaS1-casein (R2 = 0.986). Additionally, the method allowed **protein** identification and determination of the **phosphorylation** sites via MS/MS fragmentation.

L126 ANSWER 24 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999359447 EMBASE
TITLE: Identification of flow-dependent endothelial nitric-oxide synthase phosphorylation sites by mass spectrometry and regulation of phosphorylation and nitric oxide production by the phosphatidylinositol 3-kinase inhibitor LY294002.
AUTHOR: Gallis B.; Corthals G.L.; Goodlett D.R.; Ueba H.; Kim F.; Presnell S.R.; Figeys D.; Harrison D.G.; Berk B.C.; Aebersold R.; Corson M.A.
CORPORATE SOURCE: B. Gallis, Division of Cardiology, Box 359748, University of Washington, Seattle, WA 98195, United States. bgallis@u.washington.edu
SOURCE: Journal of Biological Chemistry, (15 Oct 1999) 274/42 (30101-30108).
Refs: 59
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Endothelial cells release nitric oxide (NO) acutely in response to increased laminar fluid shear stress, and the increase is correlated with enhanced phosphorylation of endothelial nitric-oxide synthase (eNOS). Phosphoamino acid analysis of eNOS from bovine aortic endothelial cells labeled with [32P]orthophosphate demonstrated that only phosphoserine was present in eNOS under both static and flow conditions. Fluid shear stress induced phosphate incorporation into two specific eNOS tryptic peptides as early as 30 s after initiation of flow. The flow-induced tryptic phosphopeptides were enriched, separated by capillary electrophoresis with intermittent voltage drops, also known as 'peak parking,' and analyzed by collision-induced dissociation in a tandem mass spectrometer. Two phosphopeptide sequences determined by tandem mass spectrometry, TQpSFSLQER and KLQTRPpSPGPPPAEQLLSQAR, were confirmed as the two flow-dependent phosphopeptides by co-migration with synthetic phosphopeptides. Because the sequence (RIR)TQpSFSLQER contains a consensus

substrate site for protein kinase B (PKB or Akt), we demonstrated that LY294002, an inhibitor of the upstream activator of PKB, phosphatidylinositol 3-kinase, inhibited flow- induced eNOS phosphorylation by 97% and NO production by 68%. Finally, PKB phosphorylated eNOS in vitro at the same site phosphorylated in the cell and increased eNOS enzymatic activity by 15-20-fold.

L126 ANSWER 25 OF 35 MEDLINE
ACCESSION NUMBER: 1999282192 MEDLINE
DOCUMENT NUMBER: 99282192 PubMed ID: 10353839
TITLE: Probing the nucleotide-binding site of Escherichia coli succinyl-CoA synthetase.
AUTHOR: Joyce M A; Fraser M E; Brownie E R; James M N; Bridger W A; Wolodko W T
CORPORATE SOURCE: Department of Biochemistry, University of Alberta, Edmonton, Canada.
SOURCE: BIOCHEMISTRY, (1999 Jun 1) 38 (22) 7273-83.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 19990712
Entered Medline: 19990623

AB Succinyl-CoA synthetase (SCS) catalyzes the reversible interchange of purine nucleoside diphosphate, succinyl-CoA, and Pi with purine nucleoside triphosphate, succinate, and CoA via a phosphorylated histidine (H246alpha) intermediate. Two potential nucleotide-binding sites were predicted in the beta-subunit, and have been differentiated by photoaffinity labeling with 8-N3-ATP and by site-directed mutagenesis. It was demonstrated that 8-N3-ATP is a suitable analogue for probing the nucleotide-binding site of SCS. Two tryptic peptides from the N-terminal domain of the beta-subunit were labeled with 8-N3-ATP. These corresponded to residues 107-119beta and 121-146beta, two regions lying along one side of an ATP-grasp fold. A mutant protein with changes on the opposite side of the fold (G53betaV/R54betaE) was unable to be phosphorylated using ATP or GTP, but could be phosphorylated by succinyl-CoA and Pi. A mutant protein designed to probe nucleotide specificity (P20betaQ) had a Km(app) for GTP that was more than 5 times lower than that of wild-type SCS, whereas parameters for the other substrates remained unchanged. Mutations of residues in the C-terminal domain of the beta-subunit designed to distrupt one loop of the Rossmann fold (I322betaA, and R324betaN/D326betaA) had the greatest effect on the binding of succinate and CoA. They did not disrupt the phosphorylation of SCS with nucleotides. It was concluded that the nucleotide-binding site is located in the N-terminal domain of the beta-subunit. This implies that there are two active sites approximately 35 A apart, and that the H246alpha loop moves between them during catalysis.

L126 ANSWER 26 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:340739 BIOSIS
DOCUMENT NUMBER: PREV199900340739
TITLE: Accurate quantitation of **protein** expression and site-specific **phosphorylation**.
AUTHOR(S): Oda, Y.; Huang, K.; Cross, F. R.; Cowburn, D.; Chait, B. T. (1)
CORPORATE SOURCE: (1) Rockefeller University, 1230 York Avenue, New York, NY, 10021 USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (June 8, 1999) Vol. 96, No. 12, pp. 6591-6596.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A **mass spectrometry**-based method is described for simultaneous identification and quantitation of individual proteins and for determining changes in the levels of modifications at specific sites on individual proteins. Accurate quantitation is achieved through the use of whole-cell stable **isotope labeling**. This approach was applied to the detection of abundance differences of proteins present in wild-type versus mutant cell populations and to the identification of in vivo **phosphorylation** sites in the PAK-related yeast Ste20 protein kinase that depend specifically on the G1 cyclin Cln2. The present method is general and affords a quantitative description of cellular differences at the level of protein expression and modification, thus providing information that is critical to the understanding of complex biological phenomena.

L126 ANSWER 27 OF 35 MEDLINE
ACCESSION NUMBER: 1999459232 MEDLINE
DOCUMENT NUMBER: 99459232 PubMed ID: 10527502
TITLE: Radiolabeling of receptor ligands by chemical incorporation of phosphorylation sites.
AUTHOR: Inglese J; Glickman J F
CORPORATE SOURCE: Pharmacopeia Inc., CN5350, Princeton, New Jersey, 08543, USA.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Oct 1) 274 (1) 104-9.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991223
AB A chemical reagent, N-acetyl-cys((succinimidyl-6-(thioacetyl)amino) hexanoate)-ser-arg-arg-ala-ser-val-tyr-amide ("phosite NHS ester"), allowing the introduction of phosphorylation sites into proteins, peptides, or small molecules, has been synthesized and characterized. The phosite reagent enables the enzymatic radiolabeling of any protein, peptide, or small molecule containing a reactive amine using [(32)P] or [(33)P]ATP and protein kinase A. The utility of the reagent has been demonstrated in cytokine and G protein-coupled radioligand receptor binding assays using whole cell and immobilized receptor formats. Use of the reagent does not require genetic manipulation of the target ligand. Copyright 1999 Academic Press.

od. 11/21/02

L126 ANSWER 28 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998198347 EMBASE
TITLE: Protein phosphorylation: Technologies for the identification of phosphoamino acids.
AUTHOR: Yan J.X.; Packer N.H.; Gooley A.A.; Williams K.L.
CORPORATE SOURCE: K.L. Williams, Macquarie Univ. Ctr. Anal. Biotech., School of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia
SOURCE: Journal of Chromatography A, (29 May 1998) 808/1-2 (23-41).

Refs: 160
ISSN: 0021-9673 CODEN: JCRAEY
PUBLISHER IDENT.: S 0021-9673(98)00115-0
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Protein phosphorylation plays a central role in many biological and biomedical phenomena. In this, while a brief overview of the occurrence and function of protein phosphorylation is given, the primary focus is on studies related to the detection and analysis of phosphorylation both in vivo and in vitro. We focus on phosphorylation of serine, threonine and tyrosine, the most commonly phosphorylated amino acids in eukaryotes. Technologies such as radiolabelling, antibody recognition, chromatographic methods (HPLC, TLC), electrophoresis, Edman sequencing and mass spectrometry are reviewed. We consider the speed, simplicity and sensitivity of tools for detection and identification of protein phosphorylation, as well as quantitation and site characterisation. The limitations of currently available methods are summarised.

L126 ANSWER 29 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998015978 EMBASE
TITLE: Properties and phosphorylation sites of baculovirus-expressed nuclear inhibitor of protein phosphatase-1 (NIPP-1).
AUTHOR: Vulsteke V.; Beullens M.; Waelkens E.; Stalmans W.; Bollen M.
CORPORATE SOURCE: M. Bollen, Afdeling Biochemie, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium. Mathieu.Bollen@med.KULeuven.ac.Be
SOURCE: Journal of Biological Chemistry, (1997) 272/52 (32972-32978).
Refs: 33
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB NIPP-1 is the RNA-binding subunit of a major species of protein phosphatase-1 in the nucleus. We have expressed nuclear inhibitor of protein phosphatase-1 (NIPP-1) in Sf9 cells, using the baculovirus-expression system. The purified recombinant protein was a potent (K(i) = 9.9 .+-. 0.3 pM) and specific inhibitor of protein phosphatase-1 and was stoichiometrically phosphorylated by protein kinases A and CK2. At physiological ionic strength, phosphorylation by these protein kinases drastically decreased the inhibitory potency of free NIPP-1. Phosphorylation of NIPP-1 in a heterodimeric complex with the catalytic subunit of protein phosphatase-1 resulted in an activation of the holoenzyme without a release of NIPP-1. Sequencing and phosphoamino acid analysis of tryptic phosphopeptides enabled us to identify Ser178 and Ser199 as the phosphorylation sites of protein kinase A, whereas Thr161 and Ser204 were phosphorylated by protein kinase CK2. These residues all conform to consensus recognition sites for phosphorylation by protein kinases A or CK2 and are clustered near a RVXF sequence that has been identified as a motif that interacts with the catalytic subunit of protein phosphatase-1.

L126 ANSWER 30 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 97195532 EMBASE
DOCUMENT NUMBER: 1997195532
TITLE: Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins.
AUTHOR: Hart G.W.
CORPORATE SOURCE: G.W. Hart, Dept. of Biochem./Molec. Genetics, University of Alabama, Schools of Medicine and Dentistry, Birmingham, AL 35294-0005, United States. gwhart@bmg.bhs.uab.edu
SOURCE: Annual Review of Biochemistry, (1997) 66/- (315-335).
Refs: 190
ISSN: 0066-4154 CODEN: ARBOAW
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Modification of Ser and Thr residues by attachment of O-linked N-acetylglucosamine [Ser(Thr)-O-GlcNAcylation] to eukaryotic nuclear and cytosolic proteins is as dynamic and possibly as abundant as Ser(Thr) phosphorylation. Known O-GlcNAcylated proteins include cytoskeletal proteins and their regulatory proteins; viral proteins; nuclear-pore, heat-shock, tumor-suppressor, and nuclear-oncogene proteins; RNA polymerase II catalytic subunit; and a multitude of transcription factors. Although functionally diverse, all of these proteins are also phosphoproteins. Most O-GlcNAcylated proteins form highly regulated multimeric associations that are dependent upon their posttranslational modifications. Evidence is mounting that O- GlcNAcylation is an important regulatory modification that may have a reciprocal relationship with O-phosphorylation and may modulate many biological processes in eukaryotes.

L126 ANSWER 31 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:166168 BIOSIS
DOCUMENT NUMBER: PREV199598180468
TITLE: Quantitative and selective fluorophore labeling of phosphoserine on peptides and proteins: Characterization at the attomole level by capillary electrophoresis and laser-induced fluorescence.
AUTHOR(S): Fadden, Patrick; Haystead, Timmothy A. J.
CORPORATE SOURCE: Dep. Pharmacol., Univ. Va., Charlottesville, VA 22908 USA
SOURCE: Analytical Biochemistry, (1995) Vol. 225, No. 1, pp. 81-88.
ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Reaction conditions were defined for the selective quantitative derivatization and fluorophore labeling of phosphoserine residues on peptides and proteins. Phosphoserine was derivatized with 1,2-**ethanedithiol** using a modification of the reaction conditions defined by R. C. Clark and J. Dijkstra (1 967) Int. J. Biochem. 11, 577-585 and H. E. Meyer, E. Hoffmann-Posorke, H. Korte, and M. G. Heilmeyer (1986) FEBS Lett. 204, 61-66 for stabilizing the phosphoamino acid during Edman degradation reactions. Following derivatization, the thiol-serine residues were coupled to fluorescence by iodoacetate reaction. Characterization by capillary zone electrophoresis and laser-induced fluorescence allowed quantitation of phosphoserine content of peptides and proteins at lt 75 amol. In three separate experiments, the overall reaction efficiency for 1,2-**ethanedithiol** derivatization of phosphoserine was estimated at 89.27 +- 2.44% (SDM). Subsequent

coupling of the derivatized serine residue with 6-iodoacetamidofluoroscein was estimated at gt 98% efficiency. Fluorescent probe tagging of phosphoamino acids on proteins and peptides offers direct quantitative evaluation of cellular **phosphorylation** states at the attomole level in tissue samples derived from plants, animals, and humans, without the use of radioisotopes, antibodies, or **mass spectrometry**.

L126 ANSWER 32 OF 35 MEDLINE
ACCESSION NUMBER: 94253184 MEDLINE
DOCUMENT NUMBER: 94253184 PubMed ID: 7515063
TITLE: Regulation of c-Fgr protein kinase by c-Src kinase (CSK) and by polycationic effectors.
AUTHOR: Ruzzene M; James P; Brunati A M; Donella-Deana A; Pinna L A
CORPORATE SOURCE: Dipartimento di Chimica Biologica, Universita di Padova, Italy.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Jun 3) 269 (22) 15885-91.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940707
Last Updated on STN: 20020420
Entered Medline: 19940630

AB The protein tyrosine kinase expressed by the protooncogene c-fgr is phosphorylated and down-regulated in vitro by the c-Src kinase (CSK). CSK catalyzed phosphorylation affects Tyr-511 of c-Fgr, homologous to Tyr-527 of c-Src and it prevents the autophosphorylation normally occurring at c-Fgr Tyr-400, homologous to c-Src Tyr-416. Polylysine, histones H1 and H2A and other polycationic proteins on the other hand stimulate c-Fgr activity while promoting enhanced autophosphorylation of both Tyr-400 and Tyr-511. Once phosphorylated at Tyr-511 and down-regulated by CSK, c-Fgr is no more susceptible to polylysine stimulation. Previous autophosphorylation (at Tyr-400) reduces c-Fgr susceptibility to down-regulation by CSK, although Tyr-511 can be still phosphorylated by it. If a more exhaustive autophosphorylation (of both Tyr-400 and Tyr-511) is performed in the presence of polylysine, c-Fgr becomes totally insensitive to CSK down-regulation. These data support the concept that down-regulation of c-Fgr by Tyr-511 phosphorylation is prevented if Tyr-400 is also phosphorylated and they are consistent with an outcompetition of phospho-Tyr-511 from the Src homology 2 domain by phospho-Tyr-400, which, in c-Fgr, is surrounded by an amino acid sequence divergent from that of the other Src-related protein tyrosine kinases.

L126 ANSWER 33 OF 35 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:544293 HCAPLUS
DOCUMENT NUMBER: 117:144293
TITLE: Transforming growth factor .beta. (TGF-.beta.) type V receptor has a TGF-.beta.-stimulated serine/threonine-specific autophosphorylation activity
AUTHOR(S): O'Grady, Pauline; Liu, Qianjin; Huang, Shuan Shian; Huang, Jung San
CORPORATE SOURCE: Sch. Med., St. Louis Univ., St. Louis, MO, 63104, USA
SOURCE: Journal of Biological Chemistry (1992), 267(29), 21033-7
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal

LANGUAGE: English
AB The transforming growth factor .beta. (TGF-.beta.) type V receptor, a newly identified high-mol.-wt. TGF-.beta. receptor (Mr .apprx. 400,000) has been purified from bovine liver plasma membranes (O'Grady, P. et al, 1991). The purified TGF-.beta. type V receptor underwent autophosphorylation at serine residues when incubated with [.gamma.-32P]ATP in the presence of 0.1% .beta.-mercaptoethanol and 2.5 mM MnCl2. This phosphorylation was stimulated by preincubation with TGF-.beta.. The preferred exogenous substrate for the Ser/Thr-specific phosphorylation activity of the type V receptor was found to be bovine casein. The TGF-.beta. type V receptor could be affinity-labeled with 5'-p-[adenine-8-14C]fluorosulfonylbenzoyl adenosine. Polylysine appeared to stimulate the autophosphorylation of the TGF-.beta. type receptor in the presence of [.gamma.-32P]ATP and the incorporation of 5'-p-[adenine-8-14C]fluorosulfonylbenzoyl adenosine into the TGF-.beta. type V receptor. The amino acid sequence anal. of the peptide fragments produced by cyanogen bromide cleavage of the purified TGF-.beta. type V receptor revealed that a peptide, namely CNBr-19, contained an amino acid sequence which shows homol. to the putative ATP binding site of the receptors for activin, the C. elegans daf-1 gene product, and TGF-.beta. type II receptor (Lin, H. Y. et al, 1992). Thus, the TGF-.beta. type V receptor is a Ser/Thr-specific protein kinase and belongs to the new class of membrane receptors assocd. with a Ser/Thr-specific protein kinase activity.

L126 ANSWER 34 OF 35 MEDLINE
ACCESSION NUMBER: 90173867 MEDLINE
DOCUMENT NUMBER: 90173867 PubMed ID: 3272343
TITLE: Refinements in oxygen-18 methodology for the study of phosphorylation mechanisms.
AUTHOR: Stempel K E; Boyer P D
CONTRACT NUMBER: GM11094 (NIGMS)
SOURCE: METHODS IN ENZYMOLOGY, (1986) 126 618-39.
Journal code: 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19970203
Entered Medline: 19900330

L126 ANSWER 35 OF 35 MEDLINE
ACCESSION NUMBER: 80039085 MEDLINE
DOCUMENT NUMBER: 80039085 PubMed ID: 40403
TITLE: Positional isotope exchange studies of enzyme mechanisms.
AUTHOR: Rose I A
SOURCE: ADVANCES IN ENZYMOLOGY AND RELATED AREAS OF MOLECULAR BIOLOGY, (1979) 50 361-95. Ref: 75
Journal code: 0337243. ISSN: 0065-258X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197912
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19980206
Entered Medline: 19791229